Inverse association between circulating vitamin D and mortality - dependent on sex and cause of death?

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Abstract: BACKGROUND AND AIMS: In various populations, vitamin D deficiency is associated with chronic diseases and mortality. We examined the association between concentration of circulating 25-hydroxyvitamin D [25(OH)D], a marker of vitamin D status, and all-cause as well as cause-specific mortality. METHODS AND RESULTS: The study included 3404 participants of the general adult Swiss population, who were recruited between November 1988 and June 1989 and followed-up until the end of 2008. Circulating 25(OH)D was measured by protein-bound assay. Cox proportional hazards regression was used to examine the association between 25(OH)D concentration and all-cause and cause-specific mortality adjusting for sex, age, season, diet, nationality, blood pressure, and smoking status. Per 10 ng/mL increase in 25(OH)D concentration, all-cause mortality decreased by 20% (HR = 0.83; 95% CI 0.74-0.92). 25(OH)D concentration was inversely associated with cardiovascular mortality in women (HR = 0.68, 95% CI 0.46-1.00 per 10 ng/mL increase), but not in men (HR = 0.97; 95% CI 0.77-1.23). In contrast, 25(OH)D concentration was inversely associated with cancer mortality in men (HR = 0.72, 95% CI 0.57-0.91 per 10 ng/mL increase), but not in women (HR = 1.14, 95% CI 0.93-1.39). Multivariate adjustment only slightly modified the 25(OH)D-mortality association. CONCLUSION: 25(OH)D was similarly inversely related to all-cause mortality in men and women. However, we observed opposite effects in women and men with respect to cardiovascular and cancer mortality.

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Abstract

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Methods: The study included 3404 participants of the general adult Swiss population, who were recruited between November 1988 and June 1989 and followed-up until the end of 2008. Circulating 25(OH)D was measured by protein-bound assay. Cox proportional hazards regression was used to examine the association between 25(OH)D concentration and all-cause and cause-specific mortality adjusting for sex, age, season, diet, nationality, blood pressure, and smoking status.

Results: Per 10 ng/mL increase in 25(OH)D concentration, all-cause mortality decreased by 20% (HR=0.83; 95% CI 0.74-0.92). 25(OH)D concentration was inversely associated with cardiovascular mortality in women (HR=0.68, 95% CI 0.46-1.00 per 10 ng/mL increase), but not in men (HR=0.97; 95% CI 0.77-1.23). In contrast, 25(OH)D concentration was inversely associated with cancer mortality in men (HR=0.72, 95% CI 0.57-0.91 per 10 ng/mL increase), but not in women (HR=1.14, 95% CI 0.93-1.39). Multivariate adjustment only slightly modified the 25(OH)D-mortality association.

Conclusion: 25(OH)D was similarly inversely related to all-cause mortality in men and women. However, we observed opposite effects in women and men with respect to cardiovascular and cancer mortality.

Introduction

Vitamin D is a steroid hormone with well-known effects on bone metabolism. Children with deficiency may suffer from rickets and adults from osteomalacia. Since several years, however, vitamin D is of interest with respect to the risk of other chronic diseases [1] because it has immunomodulation properties [2] as well as effects on cell differentiation and proliferation [3], on angiogenesis, but also on blood pressure and glucose tolerance [4]. Therefore, it was suggested that low levels of vitamin D may also increase the risk of death. Cohort studies have shown inverse association of circulating vitamin D concentration with all-cause mortality [5]. Most of these studies indicated a linear association such that highest concentrations were related to the lowest risk. However, in the largest cohort study so far among almost 250,000 Danish men and women, both low (≤ 10 nmol/L) and high (≥ 140 nmol/L) concentrations compared with 50-60 nmol/L were related to increased all-cause mortality [6].

In a recent study, an effect of vitamin D concentration on short term mortality was shown. Thus, the authors hypothesized that vitamin D might rather promote than initiate chronic diseases such as cancer and cardiovascular disease (CVD)[7]. Using data of the Swiss MONICA study, we aimed at analyzing the association between circulating levels of 25-hydroxy-vitamin D [25(OH)D], which is the most frequently used marker of vitamin D status, and mortality with a follow-up period of up to 20 years.

Methods

In a representative sample of the adult population of three Swiss cantons, 25-hydroxyvitamin D (25(OH)D) concentrations have been measured in blood samples, which have been collected between November 1988 and June 1989. The original MONICA sample that could be linked with the

Swiss National Cohort (SNC [8,9]) included 3404 participants, from which we derived mortality information up to December 31, 2008. Of these participants, 188 had to be excluded because they did not provide valid 25-hydroxyvitamin D (25(OH)D) measurements, and 25 were excluded from the analysis due to missing information on one or more other relevant variables, leading to a sample of 3191 individuals. At baseline, study participants were 25 to 74 years old.

The study participants answered a self-administered questionnaire, which assessed information on demographics, consumption of vitamin-D rich foods (meat, fish, margarine, butter, milk, and yogurt), and proxy measures of sunlight exposure (daily average time spent outdoors; vacation during the past four months). Body mass index (BMI) was calculated as measured body weight divided by squared measured body height [10]. Blood collection and measurement of 25(OH)D were described elsewhere [11]. Briefly, a venous blood sample was collected and blood samples were prepared for storage and frozen on the same day. 25(OH)D was measured several months after blood collection by protein-bound assay after extraction (acetonitrile) and purification (Sep-Pak C_{18} cartridge; Waters Associates, Milford MA) using the Amersham kit (Aylesbury, Buckinghamshire, UK). The study was conducted according to the ethical guidelines of the Swiss Academy of Medical Sciences.

Cox proportional hazards regression models were used to examine the association between 25(OH)D concentration and mortality. Time at entry was the examination date and time at exit was date of death or date of censoring, whichever came first. Vitamin D concentration was modeled continuously (per 10 ng/mL) and in quartiles based on the distribution in the cohort. In our models, we also included the following co-variables based on improvement of Akaike's information criterion (AIC) and the Bayesian information criterion (BIC): Season (December-May vs. June-November), fish/meat consumption (yes/no on the previous day), nationality (Swiss vs. non-Swiss), smoking status (never, former, or current smoker), and systolic blood pressure (continuous in mmHg). BMI did

not improve the statistical model nor change the estimates when additionally included in the models, and was, thus, not included in the final model. We examined the association of 25(OH)D concentration with all-cause mortality as well as cancer mortality and cardiovascular mortality. All models were run for the complete cohort and by sex. Tests for interaction between sex and vitamin D concentration were performed by including a variable for this interaction in the joint model for men and women. To at least partially preclude reverse causation, we additionally excluded the first five years of follow-up from the analysis. Restricted cubic spline models were used to examine possible non-linear associations between 25(OH)D concentration and mortality. Analyses were conducted with STATA 11 (Stata Corp, Texas, USA; 2009).

Results

Mean age at baseline was 47.1 years, mean follow-up time was 18.0 years. During this follow-up period, 459 of 3191 study participants died. Of these, 188 died of cancer and 122 of cardiovascular diseases.

Participants with low 25(OH)D concentrations were more likely to have participated during winter months. In contrast, the proportion of individuals who spent more the 30 minutes outdoors per day and who had been on vacation in the month prior to blood collection increased. Individuals in the top quartile of 25(OH)D concentration tended to be less often of foreign nationality, current smokers and more frequently in the upper educational level (Table 1). 10.7% of all participants had a 25(OH)D concentration < 10 ng/mL; the percentage was 7.9% among those < 40 years of age and 15.8% among those 60+ years old.

In the crude model (adjusted for age and sex), 25(OH)D concentration were inversely associated with all-cause mortality (HR=0.79; 95% CI 0.71-0.89 per 10 ng/mL increase in 25(OH)D

concentration; Table 2). Adding season, diet, and socioeconomic factors did not change the association. Only adding clinical risk factors, i.e., systolic blood pressure and smoking status, resulted in an attenuation of the association, which, however, remained statistically significant. Per 10 ng/mL increase in circulating 25(OH)D, all-cause mortality decreased by 20% (HR = 0.83; 95% CI 0.74-0.92). The results of the restricted cubic spline models did not indicate a non-linear association between 25(OH)D concentration and mortality. In a categorical model, individuals in quartiles 2, 3, and 4 had a statistically significantly lower overall mortality compared with individuals in the bottom quartile. Looking at those with a high 25(OH)D concentration of ≥ 40 ng/mL (100 nmol/L) compared with < 16 ng/mL (25 nmol/L), we observed a HR of 0.46 (95% CI 0.24-0.88). Excluding the first five years of follow-up did not materially affect the observed associations. The association between 25(OH)D concentration and all-cause mortality was statistically significant only in men (HR=0.79; 95% CI 0.68-0.91 per 10 ng/mL increase in 25(OH)D; Figure 1), but not in women (HR=0.86, 95% CI 0.73-1.02; p-interaction = 0.38), although effect estimates were similar in the categorical model for men and women (men: HR = 0.64, 95% CI 0.46-0.89; women 0.67, 0.43-1.03; top vs. bottom quartile).

Circulating 25(OH)D was inversely associated with CVD mortality in women (HR=0.68, 95% CI 0.46-1.00 per 10 ng/mL increase), but not in men (HR=0.97; 95% CI 0.77-1.23; p-interaction = 0.13). In the categorical model, HR tended to decrease with increasing quartiles of 25(OH)D concentrations in women (HR=0.42; 95% CI 0.17-1.00, top vs. bottom quartile), but not in men (HR=1.00; 95% CI 0.50-1.99; data not shown).

In contrast to CVD mortality, 25(OH)D concentrations were inversely associated with cancer mortality in men (HR=0.72, 95% CI 0.57-0.91 per 10 ng/mL), but not in women (HR=1.14, 95% CI 0.93-1.39; p-interaction=0.002; Figure 1), which was also seen in the categorical model (women HR=1.47, 95% CI 0.75-2.85, men HR=0.52, 95% CI 0.30-0.90, top vs. bottom quartile; data not shown). Among women (n cancer deaths = 72), most common cancer deaths were lung (16.6%), breast, colorectum

(9.7% each), ovaries (8.3%), and pancreas (6.9%); among men (n cancer deaths = 116), most cancer deaths were due to lung (25.8%), prostate (16.4%), colorectum (9.7%), and lymphomas (6.9%).

Discussion

In this sample from a general Swiss population, we observed an inverse association between 25(OH)D concentration and all-cause mortality. This was due to decreased cardiovascular mortality in women and decreased mortality due to cancer in men.

Our results corroborate previous findings. A meta-analysis of fourteen prospective cohort studies reported a relative risk (RR) of 0.71 (95% confidence interval [CI] 0.50-0.91) comparing highest versus lowest concentration of 25(OH)D [5]. In addition to these studies, a study from Israel, including more than 180,000 participants, observed an 85% higher all-cause mortality in participants in the bottom quartile (< 33.8 nmol/L) compared with the top quartile (> 65.2 nmol/L) [7]. The largest study so far, which included almost 250,000 Danish men and women, reported an increased all-cause mortality (RR=2.13, 95% CI 2.02-2.24) comparing low (10 nmol/L) with medium (50-60 nmol/L) concentrations. In contrast to most other studies, there was also an increase in all-cause mortality among those with very high (140 nmol/L) compared with medium concentrations of 25(OH)D (RR=1.42, 95% CI 1.31-1.53). Two other studies also observed a statistically significantly increased mortality among individuals with 25(OH)D concentrations above 97.5 nmol/L [12] and 125 nmol/L [13], respectively. In our study, we did not see such a J-shaped but rather a linear association between 25(OH)D concentration and all-cause mortality. However, the lower boundary of the top quartile was 25 ng/mL (62 nmol/L) in our cohort; only 3.4% of our study population had a concentration above 40 ng/mL (100 nmol/L). Thus, although we did not see any indication in restricted cubic spline models, we cannot exclude a positive association of very high 25(OH)D

concentrations with mortality (although the association was still inverse when comparing level ≥ 40 ng/mL with < 16 ng/mL).

Associations between circulating 25(OH)D and cause-specific mortality were reported less frequently. In our sample, we observed an inverse association with CVD mortality in women, but not in men. Inverse associations between circulating 25(OH)D and CVD have been reported in studies from the US [14-17], Finland [18], Sweden [12], and Germany [19] and a recent meta-analysis reported a pooled relative risk of 1.42 (95% CI 1.19-1.71) for CVD mortality when comparing the lowest with the highest 25(OH)D categories.[20]. Neither study reported a difference by sex. Other studies found no association between 25(OH)D concentrations and CVD mortality [21], with one, however, including only men [22]. Interestingly, a non-significant inverse association was observed among women (HR=0.83; 95% CI 0.65-1.07) in NHANES I, but not among men [21]. Our observation can be a chance finding given the small number of cases. However, we had 42 cases of CVD deaths in women and 80 in men and would have expected a statistically significant association in men rather than in women.

In contrast to CVD mortality, high circulating concentrations of 25(OH)D were related to decreased cancer mortality only in men, but not in women. Only few studies examined the association between circulating vitamin D and cancer mortality and the results are conflicting. Some studies reported inverse associations between pre-diagnostic vitamin D concentrations and all-cancer mortality [12], mortality from colorectal cancer [23,24], prostate cancer [25], and some studies have shown inverse associations between vitamin D concentration at cancer diagnosis and survival [26,27], but not all studies fully support an inverse association [22,23,28]. A cohort analysis that relied on vitamin D status indicators only, estimated that cancer mortality might decrease by 29% with an increase in 25(OH)D concentration of 25 nmol/L [29]. Other studies looked at cancer incidence instead of mortality. Strongest inverse associations were seen for colorectal cancer

incidence, e.g. in EPIC [30]. In our cohort, the most common causes of cancer death among men were prostate, lung, colorectal cancer, and lymphomas, whereas women died from lung, breast, colorectal, ovary, and pancreatic cancer. In contrast to the previous studies, in the MrOS study [22], lower 25(OH)D levels were related to a decreased risk of cancer among men and in a Swedish cohort, both high and low plasma concentrations were related to increased cancer mortality [12].

Our study has several strengths. The Swiss MONICA cohort is a sample of the general adult population of three Swiss cantons. At baseline, the study assessed a variety of factors potentially influencing the association between 25(OH)D concentration and mortality. Third, our analysis encompasses a follow-up period of up to twenty years, which is longer than in most other studies. One limitation of our study, however, is that vitamin D was only measured once at baseline. Two studies have previously reported moderate [Pearson r = 0.45; [31]] to high [Pearson r = 0.70; [29]] correlations between vitamin D measurements three years apart. In the InCHIANTI study [31], participants with vitamin D levels above and below the median at both blood draws taken three years apart had the lowest and highest rates of mortality, respectively. Consistent with this, those who were above the median at baseline and then decreased to below the median at three years had significantly higher mortality rates than those who were initially below the median and then increased above it at three years. This raises the question whether baseline vitamin D status is representative of one's future vitamin D status and whether hypovitaminosis D is merely a marker of disease and poor outcome [32]. In this respect, it is interesting to note that we observed an association between vitamin D concentration and mortality even after a follow-up period of up to 20 years. Excluding the first five years of follow-up in order to reduce the effect of reverse causation, we still observed an inverse association between circulating 25(OH)D and all-cause mortality. A recent meta-analysis has shown that cohort studies with a longer follow-up period tended to have less strong inverse associations between 25(OH)D concentration and mortality than studies with shorter follow-up [33], and Grant [34] has shown that the correlation coefficient between two vitamin D

measurements decreased from 0.7 (1-year difference) to 0.42 for a 14-year difference. However, a Norwegian study compared serum 25(OH)D concentration in participants in 1994 and in 2008 [35]. Comparing these two measurements in blood samples taken 14 years apart, they observed a correlation coefficient of 0.52. More importantly, when taking month of blood collection into account, about 50% of the study participants had a change in serum 25(OH)D of < 10 nmol/L and roughly 80% a change of < 20 nmol/L. Based on their results, the authors concluded that most individuals with low vitamin D levels are unlikely to improve their status over time. With our study design, we cannot address whether a longer follow-up leads to a "wash-out" effect such that studies with shorter follow-up time show stronger risk reductions than studies with longer follow-up time. This needs to be addressed in studies with multiple vitamin D measurements during the follow-up period.

Still, the question still remains whether a high vitamin D concentration is causally associated with chronic disease and mortality or whether vitamin D is simply a proxy for a healthy lifestyle, which cannot entirely be captured in our analysis. As previously shown, in the Swiss MONICA sample, circulating 25(OH)D was associated with season, outdoor physical activity, and diet. In our statistical models, only the addition of medical conditions and unfavorable lifestyle, i.e., systolic blood pressure and smoking, had a substantial effect on the estimates. We could not determine loss to follow-up for the entire observation time (1984-2008) but only between the 1990 and 2000 census. Nevertheless, the 4.7% (220 emigrants plus 240 individuals which could not be traced at all) lost to follow-up found for this period can be considered as low. The first National Health and Nutrition Examination Survey (NHANES I) hat a loss to follow-up of 5.6% between 1971-75 and 1982-84 [36]. Lastly, we are aware that the study's power was limited to examine associations between circulating 25(OH)D and disease-specific mortality by gender.

In conclusion, our results confirm the previously observed inverse association of circulating vitamin D levels and all-cause mortality. The observation that this inverse association is due to lower

cancer mortality only in men and lower CVD mortality only in women is new and needs confirmation. Larger studies with multiple measurements of 25(OH)D concentration are warranted in order to determine whether there is indeed a sex specificity in the association between 25(OH)D and cause-specific mortality and whether there is truly a “wash-out” effect (versus reverse causation) as an explanation for stronger risk estimates in studies with shorter follow-up periods.

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Table 1. Baseline characteristics for all study participants and by quartiles of circulating 25(OH)D concentration by sex; MONICA Switzerland 1988-2008

	All	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**
25(OH)D (ng/mL)		**0 - 13.4**	**13.5 - 18.5**	**18.6 - 24.9**	**25 - 99.8**
25(OH)D (nmol/L)		**0 - 33.5**	**33.5 - 46.2**	**46.2 - 62.2**	**62.2 - 249.1**
Men (n)	1646	419	429	417	381
Age (mean, years)	47	48.3	46.4	47.1	46.3
Follow-up time (mean, years)	17.6	16.6	17.8	18.0	18.1
Mortality					
Deaths (all-cause, n)	293	109	66	64	54
Person-years (all-cause, n)	29036	6962	7644	7519	6910
Age-standardized rate (all-cause, per 100,000 py; 95% CI)	1127 (963-1291)	1379 (1072-1686)	1077 (756-1399)	986 (696-1276)	899 (603-1195)
Deaths (cardiovascular diseases, n)	80	21	15	31	13
Deaths (cancer, n)	116	44	29	23	20
Winter season (%)	75.8	83.5	78.6	73.6	66.4
Fish/meat consumption on previous day (%)	89.5	89.3	90.4	87.8	90.6
Foreign nationality (%)	22.1	29.6	24.0	19.4	14.4
Smoking status					
Current smoking (%)	32.6	46.8	31.5	29.3	22.1
occasional smoker (%)	3.2	2.9	3.7	2.9	3.4
non smoker (%)	64.2	50.4	64.8	67.9	74.5
Blood pressure (mean systolic, mmHg)	131	133	132	129	130

Women (n)	1545	382	368	381	414
Age (mean, years)	47.2	48.9	47.6	46.5	45.7
Follow-up time (mean, years)	18.4	18.2	18.5	18.3	18.8
Mortality					
Deaths (all-cause, n)	166	58	37	34	37
Person-years (all-cause, n)	28492	6954	6796	6973	7768
Age-standardized rate (all-cause, per 100,000 py; 95% CI)	656 (541-771)	798 (559-1037)	544 (353-735)	768 (371-1166)	487 (312-661)
Deaths (cardiovascular disease, n)	42	19	10	6	7
Deaths (cancer, n)	72	16	17	16	23
Winter season (%)	76.6	85.6	82.9	70.3	68.4
Fish/meat consumption on previous day (%)	83.8	84.3	84.5	81.6	84.8
Foreign nationality (%)	15.2	23.3	13.9	12.9	10.9
Smoking status					
Current smoking (%)	25.9	29.6	27.5	22.6	24.2
occasional smoker (%)	3.6	1.8	3.0	3.7	5.6
non smoker (%)	67.3	68.6	69.6	73.8	70.3
Blood pressure (mean systolic, mmHg)	125	128	127	124	122

Table 2. Association between circulating vitamin D concentration and all-cause, cardiovascular disease and cancer mortality in MONICA, 1988-2008

	Total follow-up period		Without first 5 years of follow-up	
	N cases	**HR (95% CI)**	**N cases**	**HR (95% CI)**
All-cause mortality				
Adjusted for age & sex		0.79 (0.71-0.89)		0.82 (0.73-0.91)
Model 1 (sunlight exposure)		0.79 (0.71-0.88)		0.81 (0.72-0.90)
Model 2 (Model 1 + diet)		0.79 (0.71-0.88)		0.81 (0.72-0.91)
Model 3 (Model 2 + sociodemographic factors)		0.79 (0.70-0.88)		0.81 (0.72-0.90)
Model 4 (Model 3 + clinical risk factors)		0.83 (0.74-0.92)		0.85 (0.76-0.94)
Model 4 with Vitamin D quartiles				
Q1 (0 – 13.4 ng/mL)	167	1.00 (Ref.)	141	1.00 (Ref.)
Q2 (13.5 – 18.5 ng/mL)	103	0.69 (0.54-0.88)	98	0.77 (0.59-1.00)
Q3 (18.6 – 24.9 ng/mL)	98	0.68 (0.52-0.87)	93	0.76 (0.58-0.99)
Q4 (25 – 99.8 ng/mL)	91	0.67 (0.52-0.87)	81	0.70 (0.53-0.92)
Cardiovascular disease mortality				
Adjusted for age & sex		0.87 (0.71-1.06)		0.93 (0.76-1.14)
Model 1 (sunlight exposure)		0.87 (0.71-1.07)		0.93 (0.76-1.13)
Model 2 (Model 1 + diet)		0.88 (0.72-1.07)		0.93 (0.76-1.14)
Model 3 (Model 2 + sociodemographic factors)		0.86 (0.70-1.06)		0.92 (0.74-1.13)
Model 4 (Model 3 + clinical risk factors)		0.89 (0.73-1.08)		0.94 (0.77-1.15)
Model 4 with Vitamin D quartiles				
Q1 (0 – 13.4 ng/mL)	40	1.00 (Ref.)	33	1.00 (Ref.)
Q2 (13.5 – 18.5 ng/mL)	25	0.68 (0.41-1.13)	22	0.71 (0.42-1.23)
Q3 (18.6 – 24.9 ng/mL)	37	1.05 (0.67-1.66)	37	1.27 (0.79-2.04)
Q4 (25 – 99.8 ng/mL)	20	0.60 (0.35-1.04)	20	0.71 (0.41-1.25)
Cancer mortality				
Adjusted for age & sex		0.89 (0.76-1.05)		0.87 (0.73-1.03)

Model 1 (sunlight exposure)		0.88 (0.74-1.03)		0.85 (0.71-1.01)
Model 2 (Model 1 + diet)		0.88 (0.75-1.03)		0.85 (0.71-1.01)
Model 3 (Model 2 + sociodemographic factors)		0.87 (0.74-1.03)		0.84 (0.71-1.01)
Model 4 (Model 3 + clinical risk factors)		0.92 (0.78-1.07)		0.89 (0.75-1.06)
Model 4 with Vitamin D quartiles				
Q1 (0 – 13.4 ng/mL)	60	1.00 (Ref.)	51	1.00 (Ref.)
Q2 (13.5 – 18.5 ng/mL)	46	0.85 (0.57-1.24)	45	0.96 (0.64-1.44)
Q3 (18.6 – 24.9 ng/mL)	39	0.73 (0.49-1.10)	35	0.77 (0.50-1.19)
Q4 (25 – 99.8 ng/mL)	43	0.86 (0.58-1.28)	35	0.81 (0.52-1.26)

Model 2 (diet): adjusted for intake of fish and meat (eaten the previous day or not)

Model 3 (sociodemographic factors): nationality

Model 4 (clinical risk factors): systolic blood pressure (in mmHg), smoking status (smoker, non-smoker, occasional smoker)

Figure 1. Association between circulating 25(OH)D concentration with all-cause, CVD and cancer mortality by sex; HR per 10 ng/mL increase of 25(OH)D concentration, adjusted for age, sex, sunlight exposure, intake of fish and meat, nationality, systolic blood pressure, and smoking status

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